

# Renewal theory for single-molecule systems with multiple reaction channels

A. M. Berezhkovskii<sup>a)</sup>*Mathematical and Statistical Computing Laboratory, Division of Computational Bioscience, Center for Information Technology, National Institutes of Health, Bethesda, Maryland 20892, USA*

(Received 29 September 2010; accepted 13 January 2011; published online 18 February 2011)

Some single-molecule systems share a common feature: the system performs different cycles returning after each cycle to the same state. In such systems we deal with renewal processes. Examples include (1) single-molecule enzymatic reactions, (2) membrane transport through single-occupancy channels, (3) single-molecule fluorescence spectroscopy, and (4) motion of molecular motors. The paper is focused on the analysis of such systems by means of the renewal theory. To be more specific, the theory of renewal processes is used to study multivariate distribution functions of the numbers of different events in a given observation time. Our main results are simple formulas derived for the Laplace transforms of the distribution functions. General results are illustrated by consideration of several examples. [doi:[10.1063/1.3551506](https://doi.org/10.1063/1.3551506)]

## I. INTRODUCTION

Some single-molecule systems share a common feature: the system performs different cycles returning after each cycle to the same state. A trajectory of such a system in the space of its possible discrete states can be considered as a set of different “loops” that start from the same “initial” state. Each loop is a random walk among a subset of the states of the system that ends with the return to the initial state. The final step of the random walk that closes the loop bringing the system back to the initial state will be referred to as an event. Transitions that close loops of different types will be considered as different events. We will assume that the system has no memory in the sense that after a loop is completed, and the system returns to the initial state, it chooses the type of the next loop randomly and independently of the prehistory. In such systems we deal with renewal processes. Examples of such systems include (1) single-molecule enzymatic reactions, (2) transport through single occupancy membrane channels, (3) single-molecule fluorescence spectroscopy, and (4) transport of molecular motors (see Refs. 1–12 and references therein).

The numbers of loops of different types in a given observation time provide rather detailed information about the system. These numbers are random variables described by a multivariate distribution function that parametrically depends on the observation time  $t$ . Such distribution functions are in the focus of the present study. Formally we deal with renewal processes<sup>13,14</sup> with events of different types. Considering each type of events as a different reaction channel, we will use the term multichannel renewal processes. Our approach to the problem is based on the fact that the distribution functions of interest are nothing else than the probabilities that corresponding sets of events have happened in time  $t$ . Main results of the paper are simple formulas for the Laplace transforms of the multivariate distribution functions,

Eqs. (2.16), (2.17), (2.23), and (2.25). The former correspond to the situation when the observation starts just after an event has happened, and the system came back to the initial state, while the latter correspond to the situation when the observation begins at an arbitrary moment of time.

It may happen that the multivariate distribution function contains information about some events (loops), which are of no interest. In such a case one can eliminate this information by summing up the complete distribution function over the unimportant variables (numbers of unimportant events). As a result one obtains the distribution function of the number of events of interest only. At the same time, there is an alternative way of finding this distribution function. The way includes two steps. First, one has to eliminate unimportant events from the initial formulation of the problem, i.e., to reformulate the initial problem in terms of the events of interest only. After that one can find the desired distribution function using the results derived in the paper.

For example, in the simplest model of the enzymatic reaction,  $E + S \rightleftharpoons ES \rightarrow E + P$ , substrate  $S$  binds to enzyme  $E$ , and then enzyme–substrate complex  $ES$  decays forming the free enzyme and either product  $P$  or substrate  $S$ . In this example, according to the definitions above, events are decays of the enzyme–substrate complex leading to the recovery of the enzyme, and there are two types of the events (loops). Results presented in the paper allow one to find (i) the Laplace transform of the distribution function of the numbers of events of both types in time  $t$ , as well as (ii) the Laplace transform of the distribution function of the number of substrate molecules converted into products in time  $t$  by a single enzyme. Detailed discussion of this example is given in Sec. III, where more complex models of enzymatic reactions are also considered. These models describe the effect of the inhibitor, competition of substrates of different types for the enzyme, as well as reversible enzymatic reactions.

The outline of the paper is as follows. The general theory is developed in Sec. II. After that several illustrative examples of increasing complexity are considered in Sec. III. In

<sup>a)</sup>Electronic mail: berezh@mail.nih.gov.

Sec. IV we summarize the results obtained in the paper and briefly discuss the relation between the multivariate distribution functions and the propagator of a corresponding unidirectional random walk on a multidimensional lattice.

## II. THEORY

Consider a multichannel renewal process with events/loops of  $m$  different types. Let  $W_1, W_2, \dots, W_m$  be the probabilities of realization of each type of the events

$$W_1 + W_2 + \dots + W_m = 1, \quad (2.1)$$

and  $\varphi_i(t)$  be the probability density of the waiting time for an event of type  $i$  counted from the moment  $t = 0$  when a previous event had happened, and the system returned to the initial state,

$$\int_0^\infty \varphi_i(t) dt = 1, \quad i = 1, 2, \dots, m. \quad (2.2)$$

The plan of this section is as follows. First, to introduce the notations and the general idea of our approach to the problem we discuss renewal processes with one type of events in Sec. II A. Then we consider renewal processes with events of  $m$  types in Sec. II B. In both cases we assume that the observation starts just after an event has happened. In Sec. II C we generalize the results obtained in Secs. II A and II B to the case when the observation starts at an arbitrary moment of time.

### A. Renewal processes with one type of events

Consider a renewal process with one type of events assuming that the observation starts just after an event has happened. The probability density for the time interval between successive events is denoted by  $\varphi(t)$ . Let  $q_n(t)$  be the probability density for time when the  $n$ th event has happened. Then

$$q_1(t) = \varphi(t), \quad (2.3)$$

and  $q_n(t)$ ,  $n \geq 2$ , satisfies the recurrence relation

$$q_n(t) = \int_0^t q_1(t-t')q_{n-1}(t')dt' = \int_0^t \varphi(t-t')q_{n-1}(t')dt'. \quad (2.4)$$

As follows from Eqs. (2.3) and (2.4), the Laplace transform of  $q_n(t)$  is

$$\hat{q}_n(s) = \int_0^\infty e^{-st} q_n(t) dt = \hat{\varphi}(s)^n, \quad (2.5)$$

where  $\hat{\varphi}(s)$  is the Laplace transform of the probability density  $\varphi(t)$ .

Let  $P(n|t)$  be the distribution function of the number of events observed in time  $t$ , i.e., the probability that just  $n$  events

have happened in this time. The probability of no events in time  $t$ ,  $P(0|t)$ , is

$$P(0|t) = 1 - \int_0^t \varphi(t') dt'. \quad (2.6)$$

Its Laplace transform is given by

$$\hat{P}(0|s) = \frac{1}{s} [1 - \hat{\varphi}(s)]. \quad (2.7)$$

The probability  $P(n|t)$ ,  $n \geq 1$ , is the convolution of the probability density  $q_n(t)$  with the probability  $P(0|t)$ ,

$$P(n|t) = \int_0^t P(0|t-t')q_n(t')dt'. \quad (2.8)$$

Using Eqs. (2.5) and (2.7) we obtain

$$\hat{P}(n|s) = \hat{\varphi}(s)^n \hat{P}(0|s). \quad (2.9)$$

When the waiting time probability density is single-exponential,  $\varphi(t) = e^{-t/\tau}/\tau$ , where  $\tau$  is the mean time between successive events, its Laplace transform is  $\hat{\varphi}(s) = 1/(1+s\tau)$ . In this case the Laplace transform in Eq. (2.9) can be inverted leading to the Poisson distribution of the number of events observed in time  $t$ ,  $P(n|t) = (t/\tau)^n e^{-t/\tau}/(n!)$ .

### B. Renewal processes with events of $m$ types

Consider a  $m$ -channel renewal process. A series of events containing  $n_i$  events of type  $i$ ,  $i = 1, 2, \dots, m$ , is denoted by  $\{\mathbf{n}\}_m$ , where  $\mathbf{n}$  is a  $m$ -dimensional vector with the components  $(n_1, n_2, \dots, n_m)$ . Let  $q_{\{\mathbf{n}\}_m}(t)$  be the probability density for the duration of this series, i.e.,  $q_{\{\mathbf{n}\}_m}(t) dt$  is the probability that the last event of the series happens between  $t$  and  $t + dt$  assuming that the series began at  $t = 0$ . Only the duration of the series as a whole is of interest, and it does not matter how the events are ordered within the series. Let  $q_{n_i}^{(i)}(t)$  be the probability density for the time interval during which  $n_i$  events of type  $i$  have happened,  $i = 1, 2, \dots, m$ . We use this probability density with  $i = m$  to write the recurrence relation for  $q_{\{\mathbf{n}\}_m}(t)$ ,

$$q_{\{\mathbf{n}\}_m}(t) = \int_0^t q_{n_m}^{(m)}(t-t')q_{\{\mathbf{n}\}_{m-1}}(t')dt'. \quad (2.10)$$

Respectively, the Laplace transform of  $q_{\{\mathbf{n}\}_m}(t)$  is

$$\hat{q}_{\{\mathbf{n}\}_m}(s) = \hat{q}_{n_m}^{(m)}(s) \hat{q}_{\{\mathbf{n}\}_{m-1}}(s) = \prod_{i=1}^m \hat{q}_{n_i}^{(i)}(s). \quad (2.11)$$

Since  $\hat{q}_{n_i}^{(i)}(s) = \hat{\varphi}_i(s)^{n_i}$ , Eq. (2.5), we can write  $\hat{q}_{\{\mathbf{n}\}_m}(s)$  as

$$\hat{q}_{\{\mathbf{n}\}_m}(s) = \prod_{i=1}^m \hat{\varphi}_i(s)^{n_i}, \quad (2.12)$$

where  $n_1 + n_2 + \dots + n_m \geq 1$ .

Let  $P(\{\mathbf{n}\}_m|t)$  be the distribution function of the numbers of events of different types in time  $t$ . This distribution function is the probability that the series of events  $\{\mathbf{n}\}_m$  has

happened in time  $t$ . The probability of no events in time  $t$ ,  $P(\{\mathbf{0}\}_m | t)$ , is given by

$$P(\{\mathbf{0}\}_m | t) = 1 - \sum_{i=1}^m \int_0^t w_i(t') dt', \quad (2.13)$$

where  $w_i(t)$  is defined as

$$w_i(t) = W_i \varphi_i(t), \quad i = 1, 2, \dots, m, \quad (2.14)$$

where  $W_i$  are the realization probabilities introduced in the very beginning of this section. Probability  $P(\{\mathbf{n}\}_m | t)$  with  $n_1 + n_2 + \dots + n_m \geq 1$  can be written in terms of probability  $P(\{\mathbf{0}\}_m | t)$  and probability density  $q_{\{\mathbf{n}\}_m}(t)$ ,

$$P(\{\mathbf{n}\}_m | t) = \frac{(n_1 + n_2 + \dots + n_m)!}{n_1! n_2! \dots n_m!} \times \left( \prod_{i=1}^m W_i^{n_i} \right) \int_0^t P(\{\mathbf{0}\}_m | t - t') q_{\{\mathbf{n}\}_m}(t') dt'. \quad (2.15)$$

Here the factor in front of the integral is the realization probability of the series  $\{\mathbf{n}\}_m$ , whereas the integral gives the probability that duration of this series is equal to  $t$ . At  $m = 1$  the relation in Eq. (2.15) reduces to that in Eq. (2.8). The Laplace transforms of  $P(\{\mathbf{0}\}_m | t)$  and  $P(\{\mathbf{n}\}_m | t)$  with  $n_1 + n_2 + \dots + n_m \geq 1$  are given by

$$\hat{P}(\{\mathbf{0}\}_m | s) = \frac{1}{s} \left( 1 - \sum_{i=1}^m \hat{w}_i(s) \right), \quad (2.16)$$

and

$$\hat{P}(\{\mathbf{n}\}_m | s) = \frac{(n_1 + n_2 + \dots + n_m)!}{n_1! n_2! \dots n_m!} \times \left( \prod_{i=1}^m \hat{w}_i(s)^{n_i} \right) \hat{P}(\{\mathbf{0}\}_m | s). \quad (2.17)$$

Here we have used the relation in Eq. (2.12) and the definition of  $w_i(t)$  in Eq. (2.14).

The expressions in Eqs. (2.16) and (2.17) provide generalization of the results in Eqs. (2.7) and (2.9) to the case of multichannel renewal processes. They are main results of this subsection. In Sec. II C we relax the assumption that the observation begins just after an event has happened and generalize these results to the case when the observation begins at an arbitrary moment of time.

### C. Arbitrary beginning of the observation

Generalization of the results derived in Sec. II B to the case when the observation begins at an arbitrary moment of time can be performed in the conventional way.<sup>13,14</sup> First, we introduce the probability density of the waiting time until the first event of any type has happened. This probability density for the duration of the interval with no event,  $\varphi_0(t)$ , is given by

$$\varphi_0(t) = \frac{1}{\langle \tau_0 \rangle} P(\{\mathbf{0}\}_m | t), \quad (2.18)$$

where  $\langle \tau_0 \rangle$  is the mean time found for the probability density defined as the negative time derivative of  $P(\{\mathbf{0}\}_m | t)$ . Using the relation  $-dP(\{\mathbf{0}\}_m | t)/dt = \sum_{i=1}^m w_i(t)$ , we obtain

$$\langle \tau_0 \rangle = \int_0^\infty P(\{\mathbf{0}\}_m | t) dt = \sum_{i=1}^m \langle \tau_i \rangle W_i, \quad (2.19)$$

where  $\langle \tau_i \rangle$  is the first moment of the distribution characterized by the probability density  $\varphi_i(t)$ ,  $i = 1, 2, \dots, m$ ,

$$\langle \tau_i \rangle = \int_0^\infty t \varphi_i(t) dt = - \left. \frac{d\hat{\varphi}_i(s)}{ds} \right|_{s=0}. \quad (2.20)$$

The Laplace transform of  $\varphi_0(t)$  has the form

$$\hat{\varphi}_0(s) = \frac{1}{\langle \tau_0 \rangle} \hat{P}(\{\mathbf{0}\}_m | s) = \frac{1}{s \langle \tau_0 \rangle} \left( 1 - \sum_{i=1}^m \hat{w}_i(s) \right), \quad (2.21)$$

where we have used the relation in Eq. (2.16).

The probability of no events of any type in time,  $t$ , on condition that the observation begins at an arbitrary moment of time,  $P_a(\{\mathbf{0}\}_m | t)$ , is given by

$$P_a(\{\mathbf{0}\}_m | t) = 1 - \int_0^t \varphi_0(t') dt'. \quad (2.22)$$

Here and below subscript “a” is used to indicate that the observation begins at an arbitrary moment of time. The Laplace transform of  $P_a(\{\mathbf{0}\}_m | t)$  is

$$\begin{aligned} \hat{P}_a(\{\mathbf{0}\}_m | s) &= \frac{1}{s} (1 - \hat{\varphi}_0(s)) \\ &= \frac{1}{s} \left[ 1 - \frac{1}{s \langle \tau_0 \rangle} \left( 1 - \sum_{i=1}^m \hat{w}_i(s) \right) \right], \end{aligned} \quad (2.23)$$

where we have used the relation in Eq. (2.21).

The probability of a series of events  $\{\mathbf{n}\}_m$ ,  $n_1 + n_2 + \dots + n_m \geq 1$ , in time  $t$ , on condition that the observation begins at an arbitrary moment of time,  $P_a(\{\mathbf{n}\}_m | t)$ , is the convolution of the probability density  $\varphi_0(t)$  with the probability  $P(\{\mathbf{n}\}_m | t)$ ,

$$P_a(\{\mathbf{n}\}_m | t) = \int_0^t P(\{\mathbf{n}\}_m | t - t') \varphi_0(t') dt'. \quad (2.24)$$

This probability is the multivariate distribution function of the numbers of events of different types in time  $t$ . The Laplace transform of this distribution function has the form

$$\begin{aligned} \hat{P}_a(\{\mathbf{n}\}_m | s) &= \hat{P}(\{\mathbf{n}\}_m | s) \hat{\varphi}_0(s) \\ &= \frac{(n_1 + n_2 + \dots + n_m)!}{n_1! n_2! \dots n_m!} \\ &\quad \times \left( \prod_{i=1}^m \hat{w}_i(s)^{n_i} \right) \frac{1}{\langle \tau_0 \rangle} \hat{P}(\{\mathbf{0}\}_m | s)^2, \end{aligned} \quad (2.25)$$

where we have used the relations in Eqs. (2.17) and (2.21).

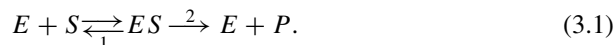
Expressions in Eqs. (2.23) and (2.25) are generalizations of the results in Eqs. (2.16) and (2.17) to the case when the observation begins at an arbitrary moment of time. In Sec. III we discuss the relation between the results derived in this section and the distribution functions that can be observed in the experiment.

### III. ILLUSTRATIVE EXAMPLES

To illustrate the general theory developed above, in this section we discuss several examples that are ordered in the increasing number of different types of events occurring in the system under consideration. For the sake of simplicity, in all examples we assume that the observation begins immediately after an event has occurred and the previous loop has completed.

#### A. Simplest model of enzymatic turnovers by single enzyme

In the simplest model of enzymatic reactions the substrate  $S$  binds to the enzyme  $E$  forming the enzyme–substrate complex  $ES$ . The complex decays either converting the substrate into the product  $P$  or not, as shown in the kinetic scheme



The numbers 1 and 2 in Eq. (3.1) show how the two decay channels of the  $ES$  complex are enumerated. Each decay of the  $ES$  complex is considered as an event. Decays in different channels are considered as events of different types. After each event the system returns to the same initial state. Here and further on the transitions indicated by the arrows are not necessarily single-exponential.

Let  $P(n_1, n_2|t)$  be the distribution function for the numbers of the two types of the events observed in time  $t$ . According to Eqs. (2.16) and (2.17) its Laplace transform is given by

$$\hat{P}(0, 0|s) = \frac{1}{s} (1 - \hat{w}_1(s) - \hat{w}_2(s)), \quad (3.2)$$

$$\hat{P}(n_1, n_2|s) = \frac{(n_1 + n_2)!}{n_1!n_2!} \hat{w}_1(s)^{n_1} \hat{w}_2(s)^{n_2} \hat{P}(0, 0|s), \quad (3.3)$$

where  $\hat{w}_i(s)$  is the Laplace transform of  $w_i(t) = W_i \varphi_i(t)$ ,  $i = 1, 2$ . We assume that  $W_i$  and  $\varphi_i(t)$  are known or can be found using available information about the reaction. This issue is explained in Appendix A where we also consider a special case when both the  $ES$  complex formation and its decay are single-exponential processes characterized by the rate constants.

Suppose one is interested in the distribution function of the number of product molecules formed in time  $t$ , which we denote by  $F(n_2|t)$ . The Laplace transform of this distribution

function,  $\hat{F}(n_2|s)$ , can be found by using Eqs. (3.2) and (3.3),

$$\hat{F}(n_2|s) = \sum_{n_1=0}^{\infty} \hat{P}(n_2, n_1|s). \quad (3.4)$$

After some manipulations one can obtain

$$\hat{F}(0|s) = \frac{1}{s} (1 - \hat{v}(s)), \quad (3.5)$$

$$\hat{F}(n|s) = \hat{v}(s)^n \hat{F}(0|s), \quad (3.6)$$

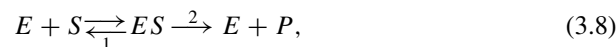
with  $\hat{v}(s)$  given by<sup>15</sup>

$$\hat{v}(s) = \frac{\hat{w}_2(s)}{1 - \hat{w}_1(s)}. \quad (3.7)$$

Expressions in Eqs. (3.5) and (3.6) describe the distribution of the number of events in the renewal process with one type of events [cf. Eqs. (2.7) and (2.9)]. The relation in Eq. (3.7) gives the Laplace transform of the interevent waiting time probability density for this effective renewal process in terms of functions  $\hat{w}_1(s)$  and  $\hat{w}_2(s)$ . In Appendix B we show how this expression can be derived starting with the kinetic scheme, Eq. (3.1).

#### B. Effect of the inhibitor

In the presence of an inhibitor ( $I$ ) the kinetic scheme in Eq. (3.1) should be modified and takes the form



In this case we deal with a three-channel renewal process in which the third channel is the formation and decay of the enzyme–inhibitor complex ( $EI$ ). Again we assume that  $W_i$  and  $\varphi_i(t)$ ,  $i = 1, 2, 3$ , are known or can be found on the basis of the kinetic scheme (see the details in Appendix C).

Let  $P(\{\mathbf{n}\}_3|t)$ ,  $\{\mathbf{n}\}_3 = (n_1, n_2, n_3)$ , be the distribution function for the numbers of events of different types observed in time  $t$ . According to Eqs. (2.16) and (2.17) its Laplace transform is given by

$$\hat{P}(\{\mathbf{0}\}_3|s) = \frac{1}{s} \left( 1 - \sum_{i=1}^3 \hat{w}_i(s) \right), \quad (3.10)$$

$$\hat{P}(\{\mathbf{n}\}_3|s) = \frac{(n_1 + n_2 + n_3)!}{n_1!n_2!n_3!} \left( \prod_{i=1}^3 \hat{w}_i(s)^{n_i} \right) \hat{P}(\{\mathbf{0}\}_3|s), \quad (3.11)$$

where  $\hat{w}_i(s)$  is the Laplace transform of  $w_i(t) = W_i \varphi_i(t)$ ,  $i = 1, 2, 3$ .

The distribution function of the number of product molecules formed in time  $t$ ,  $F(n_2|t)$ , is

$$F(n_2|t) = \sum_{n_1, n_3=0}^{\infty} P(\{\mathbf{n}\}_3|t). \quad (3.12)$$

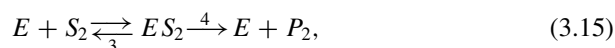
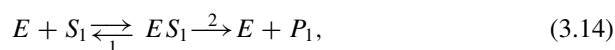
One can find its Laplace transform using Eqs. (3.10) and (3.11) and carrying out the summation. As might be expected, the result has the form given in Eqs. (3.5) and (3.6), in which  $\hat{v}(s)$  is now given by

$$\hat{v}(s) = \frac{\hat{w}_2(s)}{1 - \hat{w}_1(s) - \hat{w}_3(s)}. \quad (3.13)$$

This is the Laplace transform of the probability density of the interevent waiting time,  $v(t)$ , that describes an effective renewal process with events of one type—formation of the product molecules. One can alternatively derive the expression in Eq. (3.13) starting with the kinetic scheme, Eqs. (3.8) and (3.9). This can be done by the way similar to that discussed in Appendix B.

### C. Competing substrates

When there are two types of the substrate,  $S_1$  and  $S_2$ , which compete for the enzyme, the kinetic scheme takes the form



where  $ES_j$  and  $P_j$ ,  $j = 1, 2$ , are the enzyme–substrate complexes and products of the two types. Events are decays of the enzyme–substrate complexes. In this case we deal with four types of events/loops, which are enumerated in the kinetic scheme, Eqs. (3.14) and (3.15).

Let  $P(\{\mathbf{n}\}_4 | t)$ ,  $\{\mathbf{n}\}_4 = (n_1, n_2, n_3, n_4)$ , be the distribution function for the numbers of events of different types observed in time  $t$ . We can use Eqs. (2.16) and (2.17) to write the Laplace transform of this distribution function

$$\hat{P}(\{\mathbf{0}\}_4 | s) = \frac{1}{s} \left( 1 - \sum_{i=1}^4 \hat{w}_i(s) \right), \quad (3.16)$$

$$\begin{aligned} \hat{P}(\{\mathbf{n}\}_4 | s) &= \frac{(n_1 + n_2 + n_3 + n_4)!}{n_1! n_2! n_3! n_4!} \\ &\times \left( \prod_{i=1}^4 \hat{w}_i(s)^{n_i} \right) \hat{P}(\{\mathbf{0}\}_4 | s), \end{aligned} \quad (3.17)$$

where  $\hat{w}_i(s)$  is the Laplace transform of  $w_i(t) = W_i \varphi_i(t)$ ,  $i = 1, 2, 3, 4$ , and we assume that  $W_i$  and  $\varphi_i(t)$  are given or can be found on the basis of the kinetic scheme (see the details in Appendix D).

The function of our interest is the distribution function of the numbers of the product molecules,  $P_1$  and  $P_2$ , formed in time  $t$ ,  $F(n_2, n_4 | t)$ ,

$$F(n_2, n_4 | t) = \sum_{n_1, n_3=0}^{\infty} P(\{\mathbf{n}\}_4 | t). \quad (3.18)$$

The Laplace transform of this distribution function can be obtained by carrying out the summation and using Eqs. (3.16) and (3.17) for the Laplace transforms of the distribution functions in the right-hand side of Eq. (3.18). The result is

$$\hat{F}(0, 0 | s) = \frac{1}{s} (1 - \hat{v}_1(s) - \hat{v}_2(s)), \quad (3.19)$$

$$\hat{F}(n_2, n_4 | s) = \frac{n_2! n_4!}{(n_2 + n_4)!} \hat{v}_1(s)^{n_2} \hat{v}_2(s)^{n_4} \hat{F}(0, 0 | s), \quad (3.20)$$

where  $\hat{v}_1(s)$  and  $\hat{v}_2(s)$  are given by

$$\hat{v}_1(s) = \frac{\hat{w}_2(s)}{1 - \hat{w}_1(s) - \hat{w}_3(s)}, \quad (3.21)$$

and

$$\hat{v}_2(s) = \frac{\hat{w}_4(s)}{1 - \hat{w}_1(s) - \hat{w}_3(s)}. \quad (3.22)$$

These functions are the Laplace transforms of functions  $v_1(t)$  and  $v_2(t)$  that describe an effective (two channel) renewal process with events of two types — formation of the product molecules  $P_1$  and  $P_2$ . According to Eqs. (3.21) and (3.22) realization probabilities of these events,  $V_1$  and  $V_2$ , are given by

$$V_1 = \hat{v}_1(0) = \frac{W_2}{W_2 + W_4}, \quad (3.23)$$

and

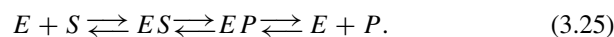
$$V_2 = \hat{v}_2(0) = \frac{W_4}{W_2 + W_4}. \quad (3.24)$$

The probability densities of their duration times, respectively, are  $v_j(t)/V_j$ ,  $j = 1, 2$ .

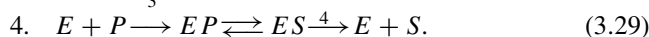
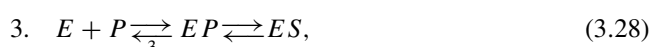
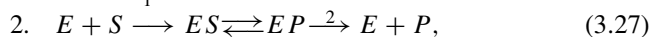
Again, there is an alternative derivation of the expressions in Eqs. (3.21)–(3.24) that starts from the kinetic scheme, Eqs. (3.14) and (3.15), and follows the way, which is similar to the one discussed in Appendix B.

### D. Single reversible enzyme

The kinetic scheme for a reversible enzymatic reaction has the form



This kinetic scheme describes loops of four different types:



Each loop begins with binding to the free enzyme and ends with the formation of the free enzyme. In the loops of types 1 and 2 the substrate binds to the enzyme while in the loops of types 3 and 4 the product binds. Events of types 2 and 4 lead to  $S \rightarrow P$  and  $P \rightarrow S$  conversions, respectively, while events of types 1 and 3 do not. Schemes 1 and 3 represent the situations when a substrate/product binds to the enzyme and then the complex decays back into the enzyme and substrate/product.

The substrate and the product play roles of the two substrates competing for the enzyme discussed in Sec. III C. Therefore, we use some results of this subsection in our further analysis. We deal with a four-channel renewal process. For such a process the Laplace transform of the distribution function of the numbers of events of different types observed



in time  $t$  is given in Eqs. (3.16) and (3.17). The Laplace transform of the distribution function of the numbers of the  $S \rightarrow P$  and  $P \rightarrow S$  conversions in time  $t$  ( $n_2$  and  $n_4$ , respectively),  $F(n_2, n_4|t)$ , is given in Eqs. (3.19) and (3.20).

We use these results to find the Laplace transform of the distribution function of the net number of substrate molecules converted into product in time  $t$ ,  $\Delta n = n_2 - n_4$ . Denoting this distribution function by  $f(\Delta n|t)$  we can write

$$f(\Delta n|t) = \sum_{n_2, n_4=0}^{\infty} \delta_{n_2-n_4, \Delta n} F(n_2, n_4|t), \quad (3.30)$$

where  $\delta_{l,m}$  is the Kronecker delta. Using the integral representation of the Kronecker delta,

$$\delta_{l,m} = \frac{1}{2\pi} \int_{-\pi}^{\pi} e^{i\theta(l-m)} d\theta, \quad (3.31)$$

the Laplace transform of  $f(\Delta n|t)$  can be written as

$$\hat{f}(\Delta n|s) = \frac{1}{2\pi} \int_{-\pi}^{\pi} \hat{f}(\theta|s) e^{-i\theta \Delta n} d\theta, \quad (3.32)$$

where  $\hat{f}(\theta|s)$  is defined as

$$\hat{f}(\theta|s) = \sum_{n_2, n_4=0}^{\infty} \hat{F}(n_2, n_4|s) e^{i\theta(n_2-n_4)}. \quad (3.33)$$

Using Eqs. (3.19) and (3.20) and performing the summation we find

$$\hat{f}(\theta|s) = \frac{1}{1 - \hat{v}_1(s)e^{i\theta} - \hat{v}_2(s)e^{-i\theta}}, \quad (3.34)$$

where  $\hat{v}_1(s)$  and  $\hat{v}_2(s)$  are given in Eqs. (3.21) and (3.22). Substituting this into Eq. (3.32) and carrying out the integration we obtain

$$\begin{aligned} \hat{f}(\Delta n|s) &= \frac{1}{\hat{k}(s) - 1} \left( \frac{\hat{v}_1(s)}{\hat{v}_2(s)} \right)^{\Delta n/2} \\ &\times \left[ \frac{2\sqrt{\hat{v}_1(s)\hat{v}_2(s)}}{\hat{k}(s)} \right]^{|\Delta n|} \hat{F}(0, 0|s), \end{aligned} \quad (3.35)$$

where

$$\hat{k}(s) = 1 + \sqrt{1 - 4\hat{v}_1(s)\hat{v}_2(s)}. \quad (3.36)$$

The result in Eq. (3.35) can be alternatively obtained if one consider a random walk, in which every  $S \rightarrow P$  and  $P \rightarrow S$  conversion corresponds to a step in the forward and backward direction, respectively.<sup>2</sup> For this random walk, functions  $v_1(t)$  and  $v_2(t)$  are products of the forward and backward step probabilities,  $V_i = \hat{v}_i(0)$ ,  $i = 1, 2$ , and the corresponding conditional step time probability densities,  $v_1(t)/V_i$ . Distribution function  $f(\Delta n|t)$  is the propagator for this random walk. Its Laplace transform can be found using the exact solution for the Laplace transform of the random walk propagator.<sup>16</sup> Eventually this leads to the result in Eq. (3.35).

#### IV. CONCLUDING REMARKS

The present paper is focused on the multivariate distribution functions of the numbers of events of different types in

time  $t$  in single-molecule systems. Our approach to the problem exploits the fact that the desired distribution functions are identical to the probabilities that corresponding sets of events have happened in time  $t$ . Main results of the present paper are the expressions for the Laplace transforms of such distribution functions given in Eqs. (2.16), (2.17), (2.23), and (2.25). The former correspond to the situation in which the observation starts immediately after an event has occurred. The latter correspond to the situation in which the observation starts at an arbitrary moment of time.

It might happen that such multivariate distribution functions provide too detailed description of the system. Keeping this in mind in Sec. III we show how unnecessary information can be eliminated, and the Laplace transforms of the distribution functions of the numbers of events of interest can be obtained from the general results. These transforms can further be used to find the Laplace transform of the distribution function of a more complex variable like the net number of the substrate molecules converted into product in time  $t$  by a single reversible enzyme considered in Sec. III D.

Finally, we note that the distribution functions discussed in the paper have another meaning. The point is that each realization of the multichannel renewal process can be considered as a realization of a nearest neighbor random walk on a multi-dimensional lattice. The random walk is unidirectional since the numbers of events cannot decrease. Thus, the distribution functions discussed above are the propagators for such random walks, and the results obtained in the paper provide exact solutions for the Laplace transforms of such propagators.

#### ACKNOWLEDGMENTS

The author is grateful to Sergey Bezrukov, Irina Gopich, Stas Shvartsman, Attila Szabo, and George Weiss for numerous enlightening discussions of different aspects of the problem and related questions. This study was supported by the Intramural Research Program of the National Institutes of Health (NIH), Center for Information Technology.

#### APPENDIX A: $W_i$ AND $\varphi_i(t)$ FOR THE KINETIC SCHEME IN EQ. (3.1)

Consider an enzyme-substrate complex formed at  $t = 0$ . Eventually this complex decays forming either the enzyme and substrate,  $ES \rightarrow E + S$  (channel 1), or the enzyme and product,  $ES \rightarrow E + P$  (channel 2). We denote the fraction of realizations that decays in channel  $i$ ,  $i = 1, 2$ , between  $t$  and  $t + dt$  by  $u_i(t)dt$ . Then the realization probability  $W_i$  is given by

$$W_i = \int_0^{\infty} u_i(t)dt = \hat{u}_i(0), \quad (A1)$$

where  $\hat{u}_i(s)$  is the Laplace transform of  $u_i(t)$ .

To find  $\varphi_i(t)$  consider a free enzyme created at  $t = 0$  immediately after a decay of an  $ES$  complex. Let  $u_E(t)$  be the probability density of the enzyme lifetime, i.e., for the time interval between the decay of a  $ES$  complex and successive formation of a new  $ES$  complex. We can write the rate of accumulation of the probability  $W_i$ ,  $w_i(t)$ ,  $i = 1, 2$ , in term of  $u_E(t)$  and  $u_i(t)$  as

$$w_i(t) = \int_0^t u_i(t-t')u_E(t')dt'. \quad (\text{A2})$$

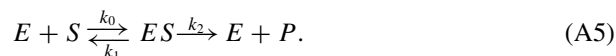
Since  $W_i = \int_0^\infty w_i(t)dt$ , we have

$$\varphi_i(t) = \frac{1}{W_i}w_i(t). \quad (\text{A3})$$

The Laplace transform of  $\varphi_i(t)$  is given by

$$\hat{\varphi}(s) = \frac{1}{W_i}\hat{u}_i(s)\hat{u}_E(s) = \frac{\hat{u}_i(s)\hat{u}_E(s)}{\hat{u}_E(0)}. \quad (\text{A4})$$

When both the  $ES$  complex formation and its decay are single-exponential, the processes is characterized by the rate constants,  $k_0$ ,  $k_1$ , and  $k_2$ , respectively, and Eq. (3.1) takes the form



In this case we have

$$u_E(t) = k_0[S]e^{-k_0[S]t}, \quad (\text{A6})$$

where  $[S]$  is the concentration of the substrate, and

$$u_i(t) = k_i e^{-kt}, \quad i = 1, 2, \quad (\text{A7})$$

where  $k = k_1 + k_2$ . Using Eqs. (A6) and (A7) we obtain

$$W_i = \frac{k_i}{k}, \quad (\text{A8})$$

and

$$\varphi_1(t) = \varphi_2(t) = \frac{k k_0 [S]}{k - k_0 [S]} (e^{-k_0 [S]t} - e^{-kt}). \quad (\text{A9})$$

## APPENDIX B: DERIVATION OF $\hat{v}(s)$ IN EQ. (3.7)

Based on the kinetic scheme in Eq. (3.1) we can write the following integral equation for the probability density  $v(t)$ :

$$v(t) = w_2(t) + \int_0^t v(t-t')w_1(t')dt'. \quad (\text{B1})$$

The two terms on the right-hand side are due to realizations in which the first decay of the  $ES$  complex either leads to the formation of the product (the first term) or not (the second term). After the Laplace transform, Eq. (B2) takes the form

$$\hat{v}(s) = \hat{w}_2(s) + \hat{v}(s)\hat{w}_1(s). \quad (\text{B2})$$

Solving this equation one recovers the expression in Eq. (3.7).

## APPENDIX C: $W_i$ AND $\varphi_i(t)$ FOR THE KINETIC SCHEME IN EQS. (3.8) AND (3.9)

Consider a free enzyme created at  $t = 0$  immediately after a decay of either an  $ES$  or an  $EI$  complex. Let  $u_E(t)$

be the probability density of the enzyme lifetime. The enzyme disappears forming a complex either with a substrate,  $E + S \rightarrow ES$ , or with an inhibitor,  $E + I \rightarrow EI$ . Therefore,  $u_E(t)$  is the sum of the corresponding terms,

$$u_E(t) = u_E^{(S)}(t) + u_E^{(I)}(t). \quad (\text{C1})$$

If the binding kinetics is single-exponential, the two terms are given by

$$u_E^{(S)}(t) = k_S[S]e^{-(k_S[S]+k_I[I])t}, \quad (\text{C2})$$

$$u_E^{(I)}(t) = k_I[I]e^{-(k_S[S]+k_I[I])t}, \quad (\text{C3})$$

where  $[S]$  and  $[I]$  are the substrate and inhibitor concentrations while  $k_S$  and  $k_I$  are the corresponding rate constants.

The enzyme–substrate complex decays forming either the enzyme and substrate,  $ES \rightarrow E + S$  (channel 1 in the kinetic scheme), or the enzyme and product,  $ES \rightarrow E + P$  (channel 2 in the kinetic scheme). We denote the fraction of realizations that decays in channel  $i$ ,  $i = 1, 2$ , between  $t$  and  $t + dt$  by  $u_i(t)dt$ , and the lifetime probability density of the inhibitor–enzyme complex by  $u_3(t)$ . With these notations, we can write functions  $w_i(t)$ ,  $i = 1, 2, 3$ , as

$$w_i(t) = \int_0^t u_i(t-t')u_E^{(S)}(t')dt', \quad i = 1, 2, \quad (\text{C4})$$

$$w_3(t) = \int_0^t u_3(t-t')u_E^{(I)}(t')dt'. \quad (\text{C5})$$

Their Laplace transforms are given by

$$\hat{w}_i(s) = \hat{u}_i(s)\hat{u}_E^{(S)}(s), \quad i = 1, 2, \quad (\text{C6})$$

$$\hat{w}_3(s) = \hat{u}_3(s)\hat{u}_E^{(I)}(s). \quad (\text{C7})$$

Using these Laplace transforms one can find the probabilities  $W_i$ ,  $W_i = \hat{w}_i(0)$ , and the Laplace transforms of the probability densities  $\varphi_i(t)$ ,  $\hat{\varphi}_i(s) = \hat{w}_i(s)/W_i$ ,  $i = 1, 2, 3$ .

## APPENDIX D: $W_i$ AND $\varphi_i(t)$ FOR THE KINETIC SCHEME IN EQS. (3.14) AND (3.15)

When two substrates compete for a free enzyme, the probability density of the enzyme lifetime,  $u_E(t)$ , is given by the two-term formula analogous to the one in Eq. (C1),

$$u_E(t) = u_E^{(S_1)}(t) + u_E^{(S_2)}(t), \quad (\text{D1})$$

where the term  $u_E^{(S_j)}(t)$  describes disappearance of the free enzyme in the process  $E + S_j \rightarrow ES_j$ ,  $j = 1, 2$ . When the kinetics of binding is single-exponential, the two terms are given by

$$u_E^{(S_j)}(t) = k_j[S_j]e^{-(k_1[S_1]+k_2[S_2])t}, \quad j = 1, 2, \quad (\text{D2})$$

where  $[S_j]$  is the concentration of the substrate of type  $j$  and  $k_j$  is the corresponding rate constant.

The enzyme–substrate complex  $ES_j$  decays forming either the enzyme and substrate,  $ES_j \rightarrow E + S_j$  (channels 1 and 3 in the kinetic scheme), or the enzyme and product,  $ES_j \rightarrow E + P_j$  (channels 2 and 4 in the kinetic scheme).

Denoting the fraction of realizations that decays in channel  $i$ ,  $i = 1, 2, 3, 4$ , between  $t$  and  $t + dt$  by  $u_i(t)dt$ , we can write functions  $w_i(t)$ ,  $i = 1, 2, 3, 4$ , as

$$w_i(t) = \int_0^t u_i(t-t')u_E^{(S_1)}(t')dt', \quad i = 1, 2, \quad (\text{D3})$$

$$w_i(t) = \int_0^t u_i(t-t')u_E^{(S_2)}(t')dt', \quad i = 3, 4. \quad (\text{D4})$$

Their Laplace transforms are given by

$$\hat{w}_i(s) = \hat{u}_i(s)\hat{u}_E^{(S_1)}(s), \quad i = 1, 2, \quad (\text{D5})$$

$$\hat{w}_i(s) = \hat{u}_i(s)\hat{u}_E^{(S_2)}(s), \quad i = 3, 4. \quad (\text{D6})$$

Using these Laplace transforms one can find the probabilities  $W_i$ ,  $W_i = \hat{w}_i(0)$ , and the Laplace transforms of the probability densities  $\varphi_i(t)$ ,  $\hat{\varphi}_i(s) = \hat{w}_i(s)/W_i$ ,  $i = 1, 2, 3, 4$ .

<sup>1</sup>J. Cao and R. J. Silbey, *J. Phys. Chem. B* **112**, 12867 (2008).

<sup>2</sup>H. Qian and X. S. Xie, *Phys. Rev. E* **74**, 010902(R) (2006).

<sup>3</sup>W. Jung, S. Yang, and J. Sung, *J. Phys. Chem. B* **114**, 9840 (2010).

<sup>4</sup>A. M. Berezhkovskii and S. M. Bezrukov, *Phys. Rev. Lett.* **100**, 038104 (2008).

<sup>5</sup>A. M. Berezhkovskii and S. M. Bezrukov, *J. Phys. Chem. B* **112**, 6228 (2008).

<sup>6</sup>A. B. Kolomeisky and S. Kotsev, *J. Chem. Phys.* **128**, 085101 (2008).

<sup>7</sup>I. V. Gopich and A. Szabo, *J. Chem. Phys.* **124**, 154712 (2006).

<sup>8</sup>I. V. Gopich and A. Szabo, in *Theory and Evaluation of Single-Molecule Signals*, edited by E. Barkai, F. L. H. Brown, M. Orrit, and H. Yang (World Scientific, River Edge, NJ, 2008), pp. 181–244.

<sup>9</sup>A. B. Kolomeisky and M. E. Fisher, *Annu. Rev. Phys. Chem.* **58**, 675 (2007).

<sup>10</sup>A. B. Kolomeisky, in *Theory and Evaluation of Single-Molecule Signals*, edited by E. Barkai, F. L. H. Brown, M. Orrit, and H. Yang (World Scientific, River Edge, NJ, 2008), pp. 313–335.

<sup>11</sup>S. M. Block, *Biophys. J.* **92**, 2986 (2007).

<sup>12</sup>C. P. Brangwynne, G. H. Koenderink, F. C. MacKintosh, and D. A. Weitz, *J. Cell Biol.* **183**, 583 (2008).

<sup>13</sup>D. R. Cox, *Renewal Theory* (Methuen, London, 1962).

<sup>14</sup>D. R. Cox and H. D. Miller, *The Theory of Stochastic Processes* (Wiley, New York, 1965).

<sup>15</sup>The author is grateful to the Referee who indicated that the formula in Eq. (3.7) can be found in Refs. 1 and 3.

<sup>16</sup>A. M. Berezhkovskii and G. H. Weiss, *J. Chem. Phys.* **128**, 044914 (2008).